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Cold Atmospheric Plasma Treatment: A Novel Method of Diabetes Mellitus Therapy: A Basic Study

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ABSTRACT: We investigate the effect of cold atmospheric plasma (CAP) therapy on diabetes in the streptozotocin (STZ)-induced diabetic rat model. In all, 30 male Wistar rats were distributed into three experimental groups: healthy, diabetic, and diabetic receiving plasma treatment. The two diabetic groups were injected with STZ to induce diabetes. Plasma treatment was performed by exposure of rats to argon plasma jet for 600 s twice a week, for 4 weeks. The results showed that the cold plasma course of therapy greatly downregulated the oxidative stress and secretion of inflammatory cytokines. Moreover, the results revealed that the glucose level of the diabetic rats decreased significantly after treatments in comparison to the diabetic control groups. Also, a decrease in advanced glycation end-product (AGE) content was achieved after therapy. The results of the study showed that cold plasma has a therapeutic effect on diabetic rats and can be considered as a noninvasive method for therapy of diabetes.

KEY WORDS: cold atmospheric plasma, diabetes, plasma medicine, oxidative stress

I. INTRODUCTION

Plasma medicine has bloomed in recent years as a new medical research field at the interface between life sciences and physics, and, over time, has become more popular and its applications are expanding.[1] Plasma is a gas-like system and a combination of charged particles, neutral species with or without excitation, and radiation (electromagnetic radiation, UV emission, visible light, and even IR radiation).[1,2] These species are chemically reactive or nonreactive, and the reactive ones can be in either radical or nonradical form such as singlet oxygen, ozone, hydroxyl radical, hydrogen peroxide, nitrite, and nitrate ions. Also, because all of these species consist of oxygen and nitrogen, they are named reactive oxygen and nitrogen species (RONS).

RONS can cause physiological or pathological effects by reacting with cell, tissue, and biological fluid constituents, including carbohydrates, lipids, and proteins.[3,4] Indeed, the biological effects of cold plasma are most likely related to change in the cell liquid environment which is induced and controlled by RONS.[5] Cold plasma has been widely

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studied for different purposes, such as skin diseases, infectious tissues, inflammatory disorders, several kinds of cancers, and the production of activated liquids.[6]

One of the most chronic diseases is diabetes mellitus (DM), which has become the leading cause of morbidity and mortality in both undeveloped and developed countries.[7] The epidemic outbreak of DM with its complications is a major crisis in global health.[8] The International Diabetes Federation (IDF) estimated that 415 million adults globally (1 in 11 adults aged 20 to 79) had diabetes mellitus in 2015.[9] This estimate is predicted to ascend to 642 million by 2040, and the most significant increases will originate from the developing countries experiencing economic shifts from low-income to middle-income levels.[10]

The main symptom of DM is hyperglycemia caused by either pancreatic β-cell autoimmune destruction and failure in the secretion of insulin (type 1 diabetes) or resistance to insulin and/or lack of insulin secretion due to a β-cell secretory defect (type 2 diabetes).[11] Insulin is a key glucoregulatory hormone. The decrease in insulin production or the reduction of insulin sensitivity results in dysregulation of glucose homeostasis that can lead to acute or chronic hyperglycemia, which is identified as the causal linkage between diabetes and longer-term microvascular and macrovascular complications.[12–14]

Reactive oxygen species (ROS), like hydrogen peroxide and superoxide anion, are essential factors involved in signal transduction in the pancreatic β-cell and have the potential to regulate the secretion of glucose-stimulated insulin.[15] Indeed, without the production of ROS or production below a specific homeostatic set point, many organisms cannot survive due to the critical physiological function of oxidants in cellular multiplication and host defense.[15] However, excessive production of ROS results in the increase of glucose and/or fatty acid oxidation and leads to disturbance of redox balance between antioxidants and oxidants, and ultimately oxidative stress. Elevated ROS can directly bind with macromolecules such as nucleic acids, proteins, and lipids, and induce oxidative damage to the cell or lead to cell death.[16] It is well-known that post-translational modifications of proteins such as oxidation, change their structures and functions. Moreover, the nonenzymatic glycation of proteins when exposed to a high glucose level, as a post-translational modification, alters the proteins' structure and function. So, hyperglycemia in a synergistic manner leads to the decrease of endogenous antioxidant defense via glycation and oxidation of protein enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) as the first-line antioxidant defense. Reduction of the enzymatic activity of antioxidants intensifies oxidative stress. Oxidative stress can be determined by measuring ROS such as hydrogen peroxide or oxidized by-products of ROS as oxidative stress biomarkers, including malondialdehyde (MDA), advanced oxidation protein products (AOPP), and low-density lipoprotein (oxLDL) as oxidized products of lipids, proteins, and low-density lipoproteins (LDL), respectively.[17]

There are many studies on the use of CAP for wound healing as wounds are one of diabetes mellitus's complications; plasma is well-known as a novel therapy for diabetic wounds.[3,18–21] However, there is no study on the effectiveness of this technique

on diabetes therapy. Although our previous studies indicated that plasma has a positive effect on the recovery of antioxidant enzyme activity and diabetes complications,[22,23] it is still unclear that plasma acts as electrical or chemical stress which induces a transient biological effect or if, during a period of time, it regulates oxidative stress, decreases inflammatory cytokine secretions and controls advanced glycation end products (AGE) content. The aim of this study was to evaluate the effect of a plasma course of therapy on diabetes in diabetic rat models via evaluation of oxidant and inflammation parameters.

II. MATERIALS

A. Assay Kits and Chemical

ELISA kits for the following materials were purchased from MyBiosource: rat AGEs (MBS261131), rat hydrogen peroxide (H_2O_2) (MBS3808898), rat advanced oxidation protein products (AOPPs) (MBS930313), rat malondialdehyde (MDA) (MBS268427), rat oxidized low-density lipoprotein (OxLDL) (MBS2501477), rat interleukin 1 alpha (IL-1α) (MBS 355416), rat interleukin interleukin 1 beta (IL-1β) (MBS825017), rat interleukin 6 (IL-6) (MBS355410), and rat tumor necrosis factor alpha (TNF-α) (MBS355371). A rat glucose assay kit (81693) was purchased from Crystal Chem. Streptozotocin (STZ) (S0130) was supplied by Sigma-Aldrich.

B. CAP Device

A high-voltage pulsed DC power supply with 10-kV amplitude and 10-kHz frequency was used for the generation of plasm. The plasma jet device consisted of a cylindrical Pyrex tube (L: 150 mm, ID: 4 mm, OD: 6 mm), high voltage, and ground electrodes. A copper rod (L: 30 mm, D: 1 mm) was used as a high voltage electrode, which was inserted into the tube. Also, a copper wire was wrapped around the tube as the ground electrode (Fig. 1).

III. METHODS

A. Optical Emission Spectroscopy

The Ocean optic HR 2000 spectrometer was used to record the optical emission spectrum of the plasma jet. The spectral range was chosen in the 200–1100-nm range with a 0.5-nm resolution. The spectrum was recorded at a 10-mm distance from the nozzle of the plasma jet and analyzed according to the atomic spectra database lines.

B. Subjects

30 male 7-week-old Wistar rats with an average weight of 210 g were bought from the Pasteur Institute in Iran. The rats were kept at a 12:12-h light-dark cycle under

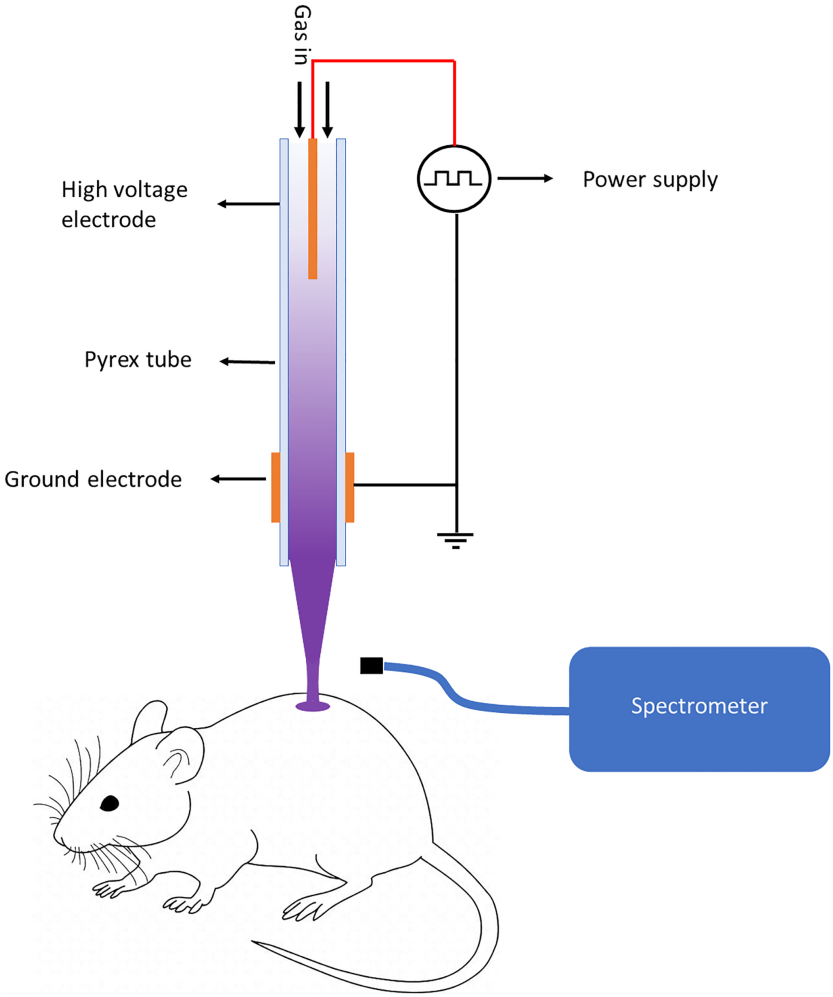

FIG. 1: Schematic of plasma jet device and plasma treatment

controlled conditions at a relative humidity of 50 ± 10% and a temperature of 23 ± 3°C, and were provided with ad libitum access to water and chow. Twenty rats were randomly selected for induction of diabetes. Streptozotocin at a dose of 50 mg kg^{-1} body weight was delivered through a single intraperitoneal injection to induce diabetes. The blood glucose levele (BGL) of the animals was checked one week after injection, and a serum glucose level of ≥ 270 mg dL^{-1} was regarded as diabetes. All rats were assigned to three 10-member groups: (1) healthy (control), (2) diabetic (group 1), and (3) diabetic receiving CAP (group 2). All animal studies were performed following international guidelines for the use and care of animals. The animal ethics review committee of the Biological Research Institute of Cognitive and Brain Sciences, Shahid Beheshti University provided all conducted studies with ethical clearance.

C. Plasma Treatment

CAP therapy was performed twice a week for 4 weeks. The rats received 600-s argon plasma jet on their back skin at each treatment (Fig. 1).

D. Sampling

One week after therapy, serum samples were gathered for measuring biochemical parameters. For this purpose, blood was collected through orbital sinus blood sampling. The samples were centrifuged at 5,000 g for 15 min, and clots were separated.

E. Oxidative Stress Analysis

Enzyme-linked immunosorbent assay (ELISA) was utilized for measuring AOPP, MDA, and oxLDL as oxidative stress biomarkers. Colorimetric method was employed to detect of H_2O_2 concentration using a quantity assay kit and the microplate reader.

F. Glucose and AGE Analysis

Determination of serum glucose of the rats was performed according to the enzymatic colorimetric method by related kit and the microplate reader. AGEs content was measured based on ELISA using the assay kit and the ELISA reader apparatus.

G. Inflammatory Factor Analysis

ELISA was utilized for measurement of inflammatory cytokines, including IL-1α, IL-1β, IL-6, and TNF-α, via detection kits, and the ELISA reader apparatus (MR-96A, Mindary Co.).

H. Statistical Analysis

All results were represented as mean ± standard deviation. We used the Kolmogorov-Smirnov test to analyze the normal distribution of data. Statistical significance was analyzed by one-way ANOVA via comparing the mean of the obtained data. After that, we used Tukey *post hoc*. p-values < 0.05 were considered significant.

IV. RESULTS

A. Optical Emission Spectroscopy

As illustrated in Fig. 2, the emission spectrum of the plasma jet contained spectral lines of argon nitrogen, oxygen, and hydroxyl radical.

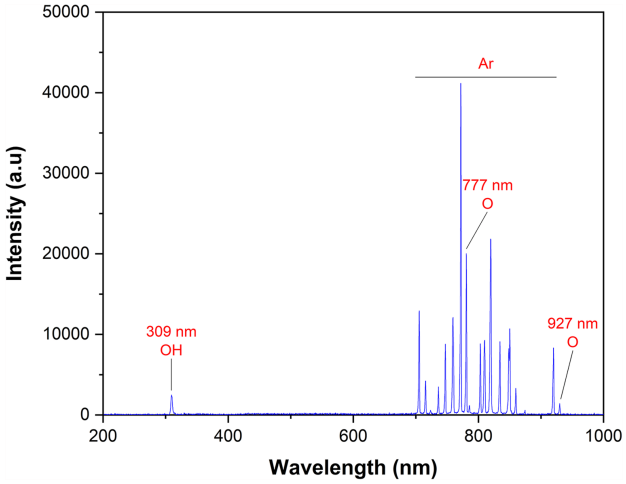

FIG. 2: Optical emission spectrum of the plasma jet

B. Effect of Plasma on Glucose, AGE, and H_2O_2 Indices

Exposure of lipids or protein to glucose induces the production of AGEs which, in the glycation of enzymatic proteins such as GPx, SOD, and catalase results in a reduction of the antioxidant defense system and finally oxidative stress. Thus, BGL has a major role in the rate of this process and the balance between oxidants and endogenous antioxidant capacity. The concentration of glucose, AGEs, and hydrogen peroxide is shown in Fig. 3, for healthy rats (control), diabetic rats (group 2), and plasma-treated diabetic rats (group 3).

According to the obtained data (Fig. 3), BGL is affected by CAP treatment such that it is reduced from 582.17 in diabetic rats to 459.00 in plasma-treated diabetic rats. Also, the results revealed that plasma is effective in the reduction of the AGEs index. Furthermore, by comparing the hydrogen peroxide concentration between groups 2 and 3, it was obvious that the plasma treatment caused a 22% decrease in the concentration of oxidant species.

C. Effect of Plasma on Oxidative Stress

Figure 4 shows the concentration of oxidative stress biomarkers, including AOPP, MDA, and oxLDL. As indicated, the concentration of AOPP in the diabetic rats significantly decreased after the plasma course of therapy. Also, a 29% reduction of the MDA index of group 3 (in comparison with group 2) was notable, showing that plasma treatment results in a decrease in lipid oxidation. As illustrated in Fig. 4, CAP treatment affects oxLDL concentration, too. The oxLDL index decreased from 22.66 in the diabetic rats to 17.33 after in the plasma-treated rats. Overall, the results show that CAP therapy causes a significant decrease in oxidative stress and relevant oxidized biomarkers.

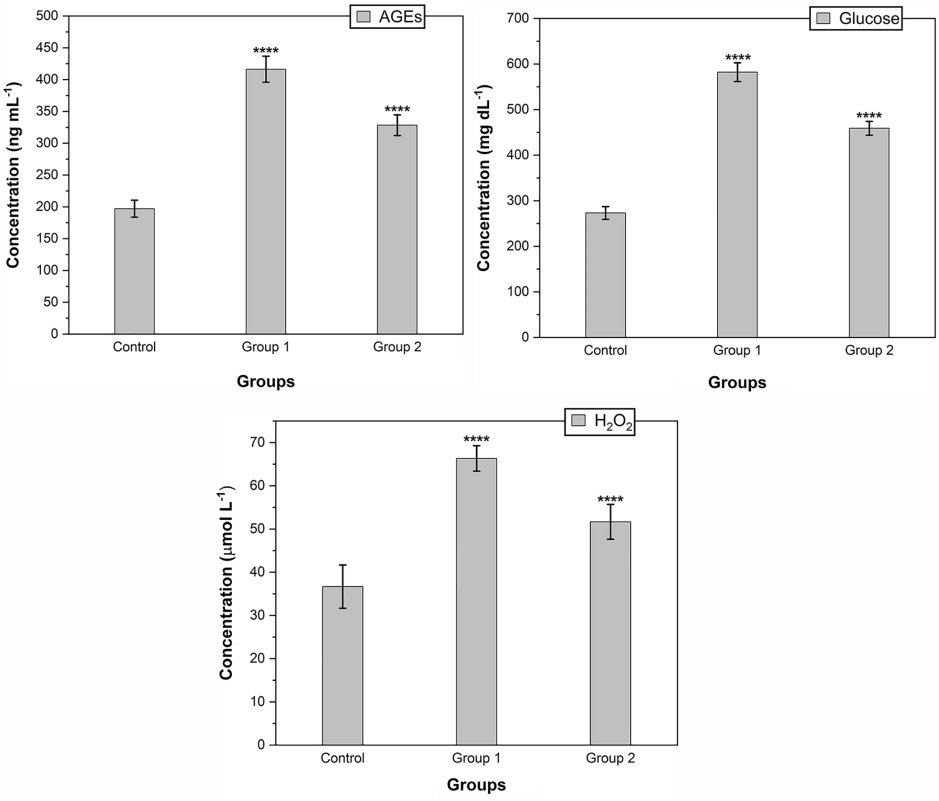

FIG. 3: Concentration of glucose, AGEs, and H_2O_2 of control, Group 1, and Group 2. *Significance of data comparing Group 2 to control and Group 3 to Group 2 (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; ****$p \leq 0.0001$).

D. Effect of Plasma on Inflammation Factors

The effect of CAP on inflammation is illustrated in Fig. 5. The results show that the inflammatory factors IL-1α, IL-1β, IL-6, and TNF-α of the treated diabetic rats were closer to those of the healthy rats than to those of the nontreated diabetic rats. Concentrations of IL-1β and IL-6 decreased approximately 21% and the concentration of IL-1α decreased from 223.33 to 176 after CAP treatment. Also, CAP treatment resulted in a 189.5 reduction of the TNF-α index. These results indicate that CAP affects diabetes-induced inflammation.

V. DISCUSSION

This study evaluated CAP therapy for diabetes. The results showed that cold plasma treatment greatly regulates oxidative stress and inflammatory cytokine secretion. Moreover, it was observed that BGL and AGE decreased after CAP.

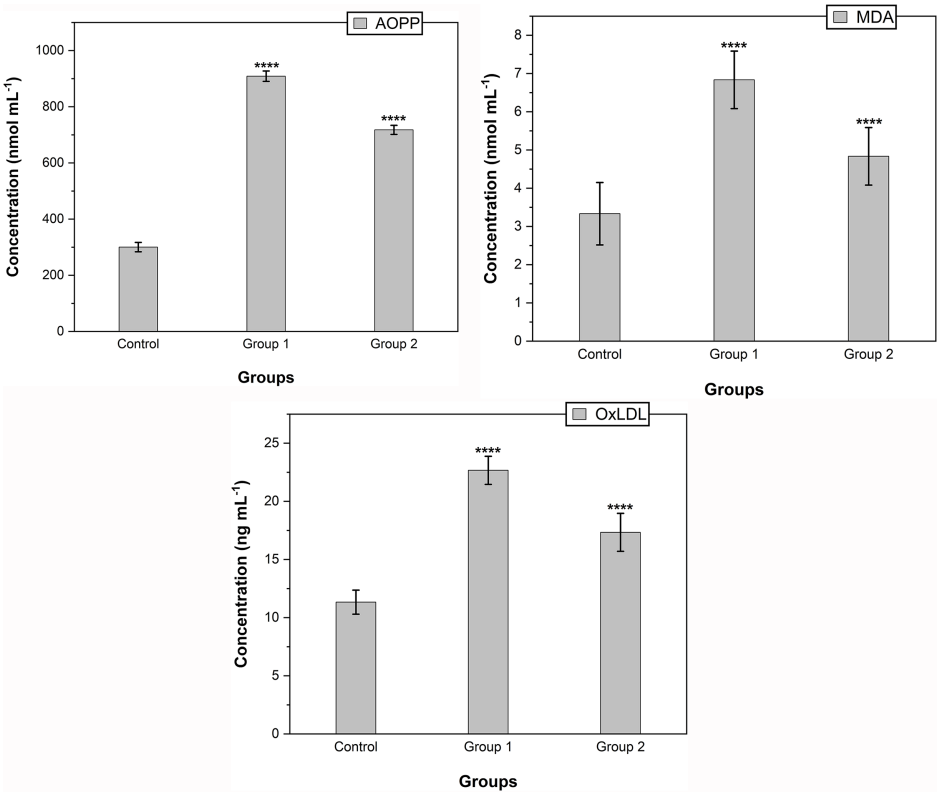

FIG. 4: Concentration of AOPP, MDA, and oxLDL of control, Group 1, and Group 2. *Significance of data comparing Group 2 to control and Group 3 to Group 2 (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; ****$p \leq 0.0001$).

It is believed that the therapeutic effects of CAP are due to RONS generated by the plasma.[24] In some applications, the therapeutic goal can be achieved by direct exposure to the plasma plume and delivery of RONS to the target surface. However, in other applications, the effects of CAP must be passed through the skin to reach the deeper layers of tissue. It is still unclear how different RONS get through the skin and reach deeper tissue layers, but there are several hypotheses. One is that the therapeutic goal is accomplished by direct penetration of RONS through the skin and its transfer to the target layer. Another involves cell-to-cell communication, in which surface layer cells are stimulated by CAP treatment to transport CAP the deeper tissue layer.[25,26] Several studies have been carried out to determine the penetration depth of RONS in tissue. Various tissue models with different plasma parameters have been employed.[27–32] The results show that long-lived RONS (H_2O_2, NO_2, and NO_3) remain in tissues after the CAP treatment, which implies that CAP has a long-term therapeutic effect.[33] Accordingly, treating the back skin of our diabetic rats with plasma could had such an effect.

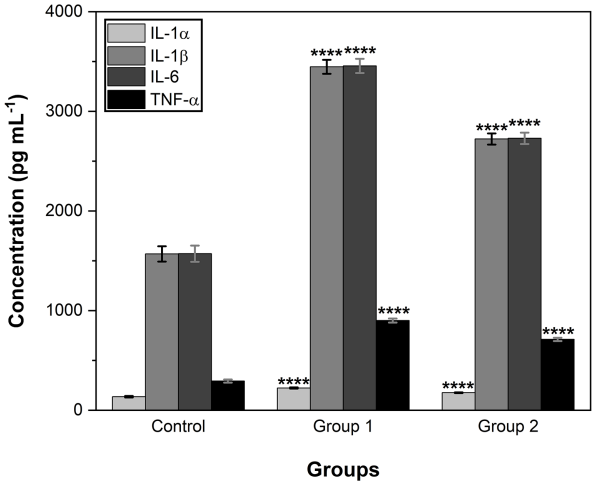

FIG. 5: Concentration of IL-1α, IL-1β, IL-6, and TNF-α of control, Group 1, and Group 2. *Significance of data comparing Group 2 to control and Group 3 to Group 2 (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; ****$p \leq 0.0001$).

In this study, it was observed that CAP therapy resulted in a decrease in oxidative stress. According to our previous study, CAP treatment can improve the antioxidant defense system through chemical modification of glycated antioxidant enzymes to restore enzymatic activity.[22] Therefore, the improvement of enzymatic activity leads to a higher neutralization rate of oxidant agents such as H_2O_2 and a decrease in oxidative stress. It has been reported that levels of antioxidant enzymes in diabetic rats, including SOD, GPx, and CAT, increased after plasma treatment.[3]

The improvement of antioxidant defense and regulation of oxidant detoxification leads to a decrease in the oxidation of proteins, lipids, and lipoproteins. In other words, a decrease in AOPP, MDA, and oxLDL as biomarkers of oxidative stress, is achieved.

Low-density lipoproteins (LDL) are highly susceptive to oxidation; ROS can oxidize the protein and lipid elements of LDL particles and produce oxLDL,[34] which has an indispensable role in the progression of endothelial inflammation and causes atherosclerosis and endothelial dysfunction.[35,36] Uptake of oxLDL by macrophages and the lectin-like oxLDL receptor-1 (LOX1) causes an increase in the formation of foam cells and the expression of the proinflammatory cytokines IL-1, IL-6, and TNF-α.[34,35,37,38] Accordingly, the decrease in of proinflammatory cytokine secretion after plasma therapy can be linked to the decrease in oxidation of LDLs because of the decline in oxidant agents or oxidative stress. Like our study, other studies have certified that plasma exposure could adjusts the quality of the inflammatory reaction via a decrease in cytokine expression.[39,40] Lee et al. verified that CAP could stop a psoriasis-like skin inflammation in mice by controlling the secretion of inflammatory cytokines.[41]

It is well-documented that RONS has an essential role in insulin physiological performance, as cellular second messengers.[42] On the other hand, it is proved that an extra

level of ROS leads body cells to resist insulin.[43] It would appear that the extension of oxidative stress along with increasing RONS modifies proteins that serve as insulin-signaling molecules, or impairs the signaling pathways of insulin.[42] Therefore, the decrease in BGL of our plasma-treated diabetic rats was probably due to the regulation of oxidative stress leading to decreasing cell resistance to insulin and thus insulin's improved performance.

The close proximity of glucose to nucleic acids, lipids, and proteins is known to lead to their long-term modification and the formation of AGEs. However, it is generally accepted AGEs and oxidative stress are strongly correlated; an increase in AGEs may result in oxidative stress and vice versa: ROS may promote AGEs.[43,44] Thus, decreasing AGEs content of treated diabetic rats can be the cause of decreasing BGL and lower oxidative stress levels.

VI. CONCLUSIONS

The results of our study show that CAP therapy can decrease the oxidative stress that has a major role in the pathogenesis of diabetes, such that the biomarkers of oxidative stress—H_2O_2, AOPP, MDA, and oxLDL—are significantly decreased. Also, our study revealed that CAP greatly affects and regulates secretions of the proinflammatory cytokines TNF-a, IL-1, and IL-6. Moreover, it showed significant decreases in BGL and AGEs due to plasma treatment—another promising point. The overall conclusion is that cold plasma has potential in diabetes treatment. However, further research is required in order to obtain a clearer understanding of the importance of the changes in pathological tissue/blood caused by CAP.

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